Effect of Silver Nano Particle Microalgae Chlorella pyrenoidosa and Dunaliella salina on Growth and Survival of Penaeus monodon Larvae

by Hermin Kusumaningrum

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Effect of Silver Nano Particle Microalgae *Chlorella pyrenoidosa* and *Dunaliella salina* on Growth and Survival of *Penaeus monodon* Larvae

Hermin Pancasakti Kusumaningrum[1*], Muhammad Zainuri[2], Indras Marhaendrajaya[3], Agus Subagio[3], Widianingsih[2], Hadi Endrawati[2], Annisa Fadillah[1], Muhammad Iskandar Zulkarnain[1], Yuvita Muliastuti[1], Imam Misbach[2]

[1]*Biotechnology Laboratory Biology Department Faculty of Science and Mathematic Diponegoro University*
[2]*Marine Laboratory, Faculty of Oceanografi and Fisheries, Diponegoro University*
[3]*Physics Department, Faculty of Science and Mathematics Diponegoro University*
Jl. Prof. H. Soedarto S.H, Tembalang, Semarang, Jawa Tengah 50275 Indonesia
Email : herminpk@live.undip.ac.id

Abstract

Penaeus monodon is one of the most important farmed crustaceans. Its also known as Asia Tiger Shrimp because its carapace and abdomen are transversely banded with red and white. The use of synthetic antibiotic in aquaculture had caused problems related to health and environmental safety. *Chlorella pyrenoidosa* and *Dunaliella salina* are photosynthetic microalgae. Silver nano particle in microalgae *C. pyrenoidosa* and *D. salina* had synthesized and showed their growth stability. They offer a potency to be exploited to supported growth and survival of shrimp larvae. The objective of the study was the application of silver nano particle in microalgae *C. pyrenoidosa* and *D. salina* on *P. monodon* larvae. The research methodology was carried out by making microalgae *C. pyrenoidosa* and *D. salina* containing silver nano particle and used as feed of shrimp larvae. Observations were made on the growth and survival of shrimp larvae compared to both microalgae and common feed. The results showed that the *P. monodon* larvae have the higher growth and survival rate with microalgae *C. pyrenoidosa* at the beginning of their growth compared to *D. salina*. However, microalgae without nanosilver and common feed showed a better result for growth and activity of shrimp larvae.

Keywords: Nanosilver, microalgae, *Penaeus monodon, Chlorella, Dunaliella*

INTRODUCTION

One of the important penaeid shrimp species in the aquaculture is the giant black tiger shrimp (*Penaeus monodon*). This is shown from the production data, in which the world shrimp dominated by *P. monodon* with a contribution of more than 50% (FAO, 2001).The growing demand for shrimp (*P. monodon*) larvae also makes it one of the leading aquaculture commodities in Indonesia. However, the potential is constrained by the presence of disease caused by microorganisms causing death of shrimp larvae up to 70% (Kusumaningrum and Zainuri, 2015). The usual way to reduce the mortality of shrimp larvae is to use antibiotics (Roque *et al*., 2001; Holmstrom *et al*., 2003; Soto-Rodríguez *et al*., 2006).This will certainly pose a health hazard to consumers. The accumulation of antibiotic residues in shrimp tissue can alter human gut flora and cause food poisoning or allergic problems (Le *et al*., 2005; Ma *et al*., 2006; Tu *et al*., 2008). The development of microalgae bionanotechnology has shown that silver nano particle integration into microalgae (SNP) has increased the potential of microalgae as antimicrobials. Silver particles in organisms are known to have good electrical conductivity, chemical stability, and catalytic activity (Balashanmugam & Kalaichelvan, 2012; Sudha *et al*., 2013; Patel *et al*., 2015; Duong *et al*., 2016; El Sheekh & El Kassas, 2016; Rajeshkumar *et al*., 2017). Natural nanoparticles in organisms has advantages compared to synthetic SNP. The use of SNP independently in medical and pharmaceutical applications also requires high cost, inefficient maintenance, as well as the contamination effects of toxic chemicals (Balashanmugam & Kalaichelvan, 2015; El Sheekh & El Kassas, 2016; Ramirez-Merida *et al*.,

*Corresponding author
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2015). Preliminary findings suggest that organic SNP are also an alternative to developing new antimicrobial agents in overcoming resistance problems (Sudha *et al*., 2013; Joyti *et al*., 2016).

There are many practical application to research about *Chlorella* and *D. salina* microalgae as a health food and dietary supplement, as well as in the pharmaceutical and cosmetic industries (Merin *et al*., 2010; Kusumaningrum & Zainuri, 2015). Microalgae has been used in the pharmaceutical industry because of the content of bioactive compounds with enormous drug potential (Dahoumane *et al*., 2017).

The biosynthesis of SNP in microalgae has been done (Dash *et al*. (2012) and El-Sheekh and El-Kassas (2016). Microalgae as photosynthetic organisms play an important role in aquatic environments, because it is not only a source of natural animal feed cultivation but also determinant of fertility and water quality. Cultivated animals such as shrimps (*Penaeus* sp.) and fish are one of the leading commodities in Indonesia and major foreign exchange contributors. The availability of cultivated shrimp larvae has decreased due to disease, limitations and rising feed prices, and the management of feed and cultivation environment that are poorly observed. Increased animal disease resistance due to the quality of waters contaminated by pathogenic microbes is a very decisive aspect in the culture of shrimp larvae because it is very influential on the production and supply availability of the supply chain. Contamination and nutrient deficiency can lead to a decrease in the production of *P. monodon* reaches 60-70%. Photosynthetic microalgae as the primary source of nutrients are needed to improve pigmentation due to their carotenoid content and the livelihood of aquaculture animals. The aquaculture requires antioxidant supplements such as carotenoids but is unable to synthesize de novo. Synthesis and characterization of SNP in *Chlorella* microalgae have been done by Annamalai & Nallamuthu (2016), but no reports have been reported on SNP application of *C. pyrenoidosa* and *D. salina* in shrimp larvae and their effect on activity and growth. Although previous study have adressed the potency of microalgae, but the influence of *C. pyrenoidosa* and *D. salina* microalgae containing SNP as feed has never been studied especially for shrimp larvae, especially with combination position as a determinant of water quality and natural food sources. The objective of the study was the application of nanosilver microalgae of *C. pyrenoidosa* and *D. salina* on *P. monodon* larvae to search their effect on its growth and survival.

MATERIAL AND METHODS

Postlarvae *P. monodon* was obtained from Brackish Water Aquaculture Development Center (BBPBAP) in Jepara Indonesia. They are maintained in containers of seawater using indoor circulation with temperatures set at 25-28^{o}C and salinity at 30-32 ‰. The container is cleaned daily.

Biosynthesis of SNP microalgae *C. pyrenoidosa* and *D. salina*

Microalgae products containing silver nanoparticles were prepared using the method according to Balashanmugam *et al*. (2015) and Patel *et al*. (2015). Microalgae culture about 100 mL of were taken in the logarithmic phase and mixed into 250 mL of 2 mM $AgNO_3$ solution. The solution was allowed to react for 1 hour. After that, 10 mL of 0.5% PAA solution were added to the solution and stirred for 2 hours using a magnetic stirrer. Characterization of the mixed solution in the form of color, UV-Vis absorption spectrum at a wavelength of 300nm and 800 nm at pH 7 after incubation at 0.5 hours; 6 hours; 24 hours; and 168 hours. This method is based on the formation of a brownish color from aqueous solution of $AgNO_3$ due to surface plasmon resonance excitation. Microalgae that showed peaks in absorbance between 400 and 450 nm were identified as microalgae containing silver nanoparticles. Determination of silver nano particle and microalgae cell containing SNP size in mixed solutions was carried out using SEM. The precipitates from the synthesis of silver nano particles microalgae were characterized by TEM for qualitative analysis in order to obtain a description of the morphology of the cell.

Analysis of growth and survival

The test shrimp larvae of growth and survival were measured after feed with SNP microalgae *C. pyrenoidosa* or *D. salina* comparing with microalgae feed and common feed as a control. The shrimp stage is selected at the post-15-day postlarvae stage (P.L.15-P.L.20), where shrimp larvae are capable of transporting, acclimatization and dispersal since the gills are fully developed (Palacios *et al*., 2004). After acclimatization, healthy shrimp larvae were

divided into four groups consisting of shrimp larvae which were given common feed (UM), SNP microalgae *C. pyrenoidosa* (CPN) or *D. salina* (DSN), SNP, microalgae *D. salina* (DS), and *C. pyrenoidosa* (CP). Each treatment consisted of three replications, each containing 10 shrimp per tank for four experiments. Every 24 hours, measurements of the growth weight of shrimp larvae and the number of live shrimp larvae were measured. This observation was carried out for 7 days

SEM analysis of microalgae silver nanoparticles *C. pyrenoidosa* and *D. salina*

Scanning electron microscopic (SEM) analysis was carried by using SEM Jeol JSM 6510 LA model. Samples of the dray material from aqueous solution of SNP and microalgae containing SNP were prepared by centrifugation at 8,000 rpm for 5 min. The pelet was dried. The SEM micrographs have been produced with magnifications 200, 3000, 5000, 10000 and 20000 x (diameters). SEMs are equipped with X-ray analytical capabilities. Thus topographic, cristallographic, and compositional information can be obtainaed rapidly, efficiently, and simultaneously from the same area.

RESULT AND DISCUSSION

The farmers generally evaluate the health status of shrimp culture by using observations on survival rates, mortality and growth, feed conversion ratio, varying sizes and changes in body and organ colors. In addition they will also observe stress levels, behavioral, physical, and digestive (FAO, 2001). Compared with production of commercial vertebrate animals, in general there is no specific criterion evaluation of shrimp health status (Bachère, 2000).

Synthesized SNP microalgae *C. pyrenoidosa* and *D. salina* are shown in Figure 1. It appeared that SNP that can be entered into cells had a varying size, i.e. 1-100 nm (Figure 2) which did not cause cell lysis. Other researchers had synthesized SNP in microalgae *C. vulgaris* showed the same result (Annamalai and Nallamuthu, 2016). However, different results obtained by Oukarroum *et al*. (2012); Minneto *et al*. (2016); Raj (2017), where the effect of silver on *D. tertiolecta* and *C. vulgaris* with silver exposure for 24-72 hours and concentrations between 0.0000229 and 10 mg.L^{-1} Silver had caused cell growth restriction, decreased chlorophyll content and cell viability. Based on the results of the study, it is known that the effect of silver exposure to microalgae that is still safe against microalga is also determined by the time of exposure of SNP with microalgae.

Effect of SNP microalgae *C. pyrenoidosa* and *D. salina* on growth of *P. monodon*

Based on the results of the study as shown in Figure 3, it appears that SNP microalgae *C. pyrenoidosa* and *D. salina* feed is able to increase the weight of shrimp larvae higher than with common feed. However, because both microalgae

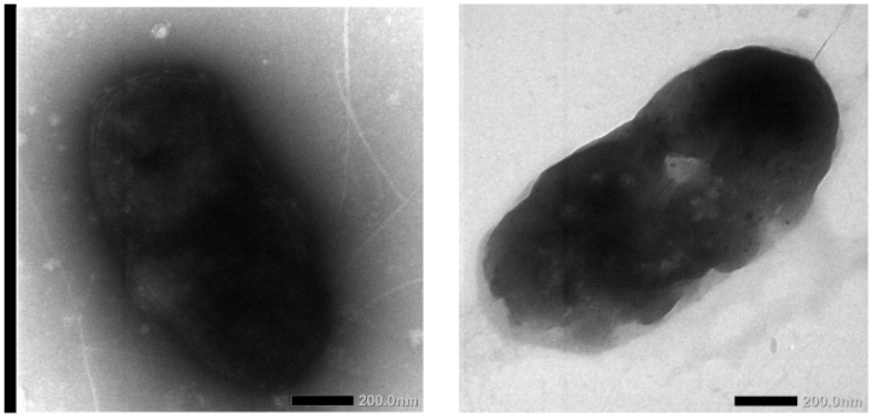

Figure 1. Microalgae *C. pyrenoidosa* (CPN) (left) and *D. salina* (DSN) (right) containing silver nano particle based on SEM observation

have a growth cycle of nine days, the weight of the shrimp larvae also decreases with the reducing number of microalgae cells. Silver is an inorganic antimicrobial widely used to reduce pathogen infection. Resistance to aquatic pathogens is generally obtained from commercially available antimicrobial agents and antibiotics. The frequently antimicrobial and antibiotics used in aquaculture to combat bacterial diseases on shrimp include oxytetracycline, florfenicol, sarafloxacin, and enrofloxacin (Roque *et al*., 2001; Soto-Rodríguez *et al*., 2006) chlorortracycline, quinolone, ciprofloxacin, norfloxacin, oxolinic acid, perfloxacin, sulfamethazine, gentamicin, and thiamulin (Holmstrom *et al*., 2003). However, Kholoud *et al*. (2010) and Dash *et al*. (2012) also found that SNP has significant adverse effects on growth and morphology on filamentous green algae in a dose-dependent manner.

Feeding SNP microalgae *C. pyrenoidosa* and *D. salina* at a concentration of 2 mM with a particle size of 1-100 nm in shrimp larvae in Figure 4 shows a high survival rate in shrimp larvae on the first to third days. It also appears that the decrease in the number of SNP microalgae after three days of cultivation was due to the longer content of silver particles in the microalgae cells which will cause the cell walls to break, releasing silver particles into the solution and reducing the survival rate of shrimp. This possibility is supported by Iravani *et al*. (2014) and Raj *et al*. (2017) in an experiment with *Drosophila melanogaster* which showed that SNPs decreased survival, longevity, ovarian size and ability to lay eggs at low doses.

The survival of shrimp larvae showed a decrease on the ninth day of cultivation except for common feed and natural feed from microalgae. One of the factors that influence shrimp activity during its growth is feeding activity. Microalgae are natural organic food for postlarvae shrimp which can reproduce quickly. Microalgae existence in water stimulate the feeding activity of shrimp (Karthik *et al*., 2015).

The feeding in the early stages of shrimp larvae in seeding is in the form of a balanced diet and nutritional supplements. This study was in agreement with other researcher which found that live food has always been a major nutrient in shrimp larvae culture (Richmond, 2004). In shrimp larvae, phytoplankton like microalgae is a major source of proteins, carbohydrates, lipids and other nutrient compounds. The growth rate of shrimp larvae is strongly related to the type of microalgae and microalgae biochemical composition used as feed (D'Souza and Loneragan, 1999). Biochemical composition of microalgae is generally influenced by cultivation conditions, light intensity, pH, temperature and nutrients (Kusumaningrum and Zainuri, 2014).

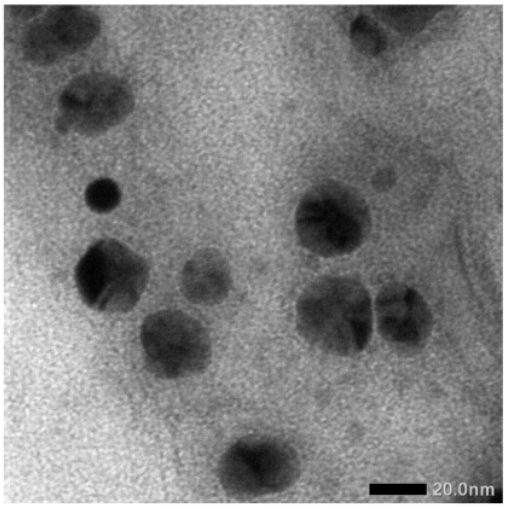

Figure 2. Silver nano particle size based on SEM observation

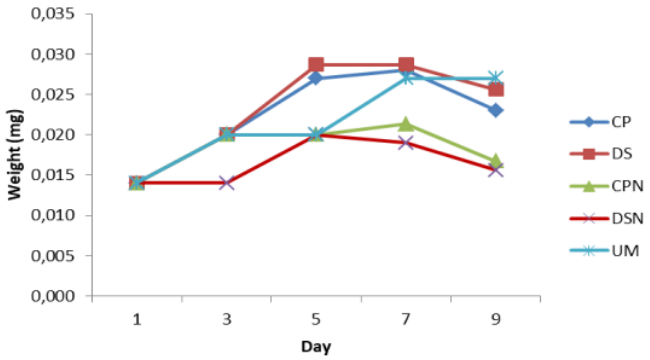

Figure 3. Average of weight of shrimp in cultivation, fed with *C. pyrenoidosa* (CP), or nanosilver *C. pyrenoidosa* (CPN). *D. salina* (DS), nanosilver *D. salina* (DSN), common feed (UM)

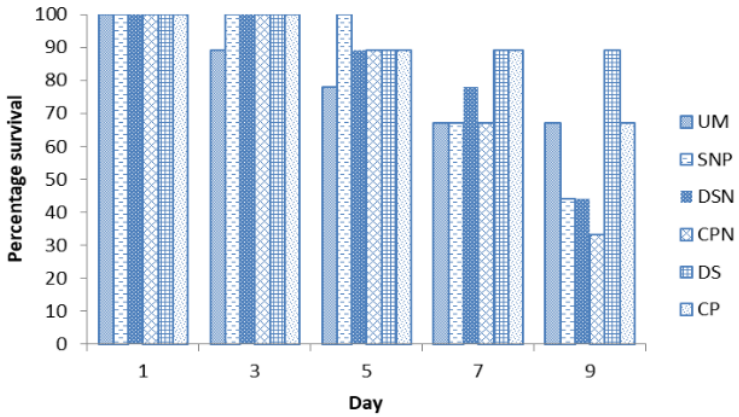

Figure 4. Percentages of shrimp larvae survival in cultivation, feed with common feed (UM), Silver Nano Particle (SNP), nanosilver *D. salina* (DSN), nanosilver *C. pyrenoidosa* (CPN), *D. salina* (DS) and *C. pyrenoidosa* (CP)

The comparison between growth and survival rates in shrimp larvae shows that although larvae weight decreases by feeding natural microalgae containing SNP and microalgae alone, their survival tends to increase with natural feeding compared to general feed. These results indicate that the use of microalgae as natural food supports the survival of shrimp larvae. The most important finding in this study was the synthesis of microalgae SNP of *C. pyrenoidosa* and *D. salina* has shown that SNP microalgae show positive effect on growth and survival of *P. monodon* larvae in early growth phase. Further research that will be developed is the use of microalgae SNPs as natural antimicrobials by considering the effect of SNP dosage, exposure time, microalgae growth media and types of microalgae.

CONCLUSION

The application of the *C. pyrenoidosa* and *D. salina* silver nanoparticles can improve growth and survival of shrimp larvae. The results also show the potential of microalgae to be used as natural agents that can reduce toxicity in the aquaculture environment, thus creating better environmental conditions in shrimp hatcheries.

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